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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
	Application Number	09/632,735
	Filing Date	August 4, 2000
	First Named Inventor	BAEZA-RAMIREZ
	Group Art Unit	1641
	Examiner Name	K. Padmanabhan
	Attorney Docket Number	2480-103

Title: **METHODS FOR DIAGNOSTIC AND/OR TREATMENT OF ANTIPHOSPHOLIPIDS ANTIBODIES-RELATED DISEASES AND DEVICES**

APPEAL BRIEF

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is an appeal from the Office Action dated January 27, 2003 in which claims 32, 35-38, 46, 48, 52-59, 91-92 and 94 were rejected as anticipated by Loizou et al, claims 32, 35-38, 46, 52-59 and 91-95 were rejected as anticipated by Stewart et al and claims 32, 35-38, 46, 48-49, 53-55, 59 and 91-95 were rejected as obvious over Ramirez et al in view of Sugi et al. A Notice of Appeal was filed on May 27, 2003, after receiving an advisory action on May 5, 2003.

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Real Party in Interest

The owner of the above-referenced patent application and the real party in interest in this appeal is the assignee, Escuela Nacional de Ciencias Biologicas, del Instituto Politechnico Nacional, located in Mexico.

Related Appeals and Interferences

Applicants are unaware of any other appeals or interferences related to the subject matter of this appeal.

Status of Claims

Claims 32, 35-38, 46, 48-49, 52-59, and 91-95 are pending and are under rejection as a result of the Office Action dated January 27, 2003. Applicants appeal from the rejection of claims 32, 35-38, 46, 48-49, 52-59, and 91-95. The appealed claims are reproduced in the appendix.

Status of Amendments

There have been no amendments filed subsequent to the last rejection of the claims.

Summary of The Invention

The invention is directed to a diagnostic method and kit for indirectly determining the presence of lipidic particles in a sample from an individual suspected of having anti-lipidic particle antibodies. The determination of lipidic particles in the membrane is a diagnostic for determining whether an individual is developing an illness associated with the presence of antiphospholipid antibodies. The invention allows diagnosis of such illnesses in circumstances

where an individual is developing such illness but does not present anti-cardiolipin, anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies.

The diagnostic method includes combining a sample, which may have anti-lipidic particle antibodies, with an antigen which is a lipidic particle immersed in a bilayer structure but itself not being part of the bilayer structure. The complex formed between the antibody and the antigen can be detected with a detectable labeled reagent for detecting binding of the anti-lipidic particle antibody to the antigen. This detection allows for the determination of the presence of anti-lipidic particle antibodies, which presence may be correlated to immune damage in cell membranes having lipidic particles.

The anti-lipidic particle antibodies are a unique type among the antiphospholipid antibodies. Anti-lipidic particle antibodies recognize as antigens lipidic particles. These lipidic-particles are unique arrangements of cardiolipin and phospholipids. Cardiolipin and other phospholipids are structurally organized in liposomes or hexagonal II arrangements which are different from lipidic particles. Cardiolipin is a phospholipid, which is associated in closed phospholipid bilayers or liposomes when found in an aqueous media (Specification Page 4, lines 22-25) (Figure A). However, cardiolipin in the presence of divalent cations changes its molecular association in said aqueous media and forms hexagonal II arrangements (Specification Page 4, lines 28-30) where cardiolipin is associated in a tubular form (Figure B). Additionally, when cardiolipin is forming liposomes together with other phospholipids such as phosphatidylcholine, and those liposomes are treated with divalent cations or drugs that produce lupus induced by drugs in humans, the formation of a different and special phospholipid arrangement (called lipidic particle) occurs (Specification Page 5, lines 6-13; page 12, lines 30-

34 and page 13, lines 1-3). This special phospholipid molecular arrangement is schematically shown in Figure C .

Thus, cardiolipin and phospholipids can be induced, under special circumstances, to form an unique structural arrangement, namely, lipidic particles. Lipidic particles are defined on page 5, lines 1-5 of the specification as "lipidic arrangements in hexagonal or micellar phases, as well as any other structural arrangement of lipids that does not form a bilayer but that is immersed in a bilayer". Additionally, "lipidic particles are formed by incubation of liposomes with an effective amount of the lipidic particle inductor agent (divalent cations: Ca or Mn, or drugs that produce lupus induced by drugs in human beings such as chlorpromazine or procainamide) at a temperature between 25 to 40°C" (Specification Page 13, line 34 to page 14, line 7). Thus, the structure of these lipidic particles immersed in a biological bilayer (a cell membrane) is very different from the structure of a cardiolipin bilayer or a hexagonal II arrangement of cardiolipin.

Therefore, the antibodies in the present invention are anti-lipidic particle antibodies. Anti-lipidic particle antibodies are not directed to an individual type of phospholipid, but recognize a lipidic particle structure containing multiple different phospholipids. Phospholipids such as cardiolipin associated in lipidic particles in the lipid bilayers of liposomes or cells are the antigens used in this invention. In consequence, the monoclonal antibodies used in the present invention specifically react with phospholipids associated in lipidic particles, and they do not react with phospholipids associated in other molecular arrangements, such as liposomes or hexagonal II arrangements. In a similar way the polyclonal antibodies detected with the present invention are antibodies against phospholipids associated in lipidic particles in liposomes or in cells (Figure C).

In one aspect, the invention comprises a diagnosis method which uses monoclonal antibodies specific to lipidic particle antigens, that respond in the same way as the anti-lipidic particle antibodies present in sera from patients with diverse illnesses associated with antiphospholipid antibodies, as set forth in claims 32, 35-38, 91-93, and 95. Further, the present invention provides a kit or diagnosis set for the detection of anti-lipidic particles antibodies in early stages of illnesses that present such antibodies in animals and in humans, as set forth in claims 46, 48-49, 52-59, and 94. The Applicants have discovered that the early stages of an illness associated with the presence of antiphospholipid antibodies can be determined with a diagnostic method, using the detection of anti-lipidic particle antibodies in circumstances where an individual does not present anti-cardiolipin antibodies, lupus anti-coagulant, anti- DNA antibodies or anti-nuclear antibodies.

Specifically, independent claim 32 is directed to a diagnostic method for indirectly determining the presence of lipidic particles in cell membranes. The method comprises removing a sample from an individual, combining the sample with an antigen having lipidic particles, adding to this mixture a detectable-labeled reagent for detecting binding of an anti-lipidic particle antibody to the antigen, detecting the presence of anti-lipidic particle antibodies in the sample and correlating the presence of anti-lipidic particle antibodies with immune damage in the cell membrane. Dependent claims 35-38 are directed to the method of claim 32 where the detectable labeled reagent comprises a detectable labeled polyvalent anti-human imminoglobulin second antibodies and reagents. Independent claim 46 is directed to a kit for use in a diagnostic method as described above. Dependent claims 48 and 49 are directed to the kit of claim 46, wherein the antigens comprise liposomes or neoplastic cells. Dependent claim 52 is directed to the kit of

claim 46, wherein the buffer solution has a pH of 7.0 to 7.4. Dependent claim 53 is directed to the kit of claim 46, wherein the sample is selected from the group consisting of serum and plasma. Dependent claims 54-56, and 59 are directed to the kits of claim 46, wherein the detectable labeled reagent comprises a detectable labeled polyvalent anti-human immunoglobulin second antibodies and reagents. Dependent claims 57 and 58 are directed to the kits of claims 46, wherein the kit further includes a blocking solution and an anti-lipidic particle antibody respectively. Dependent claims 91 and 92 are directed to the methods of claim 32, wherein the sample of the individual comprises an antibody porter, and wherein the antibody porter comprises plasma or serum respectively. Dependent claims 93 and 95 are directed to the methods of claim 32, wherein the lipidic particles have a structural arrangement which is immersed in a bilayer structure, and wherein the structural arrangement is selected from the group consisting of arrangements in hexagonal II or micellar phases respectively. Finally, dependent claim 94 is directed to a kit of claim 46, wherein the antigen is selected from the group consisting of erythrocytes, leukocytes and plaquettes.

Issues

The following issues are presented by this appeal:

1) Whether the subject matter of claims 32, 35-38, 46, 48, 52-59, 91-92 and 94 is novel and not anticipated by Loizou et al under by 35 U.S.C. §102(b).

2) Whether the subject matter of claims 32, 35-38, 46, 52-59, and 91-95 is novel and not anticipated by Stewart et al. under 35 U.S.C. § 102(b).

3) Whether the subject matter of claims 32, 35-38, 46, 48-49, 53-55, 59, and 91-95 is non-obvious over Ramirez et al in view of Sugi et al under 35 U.S.C. § 103(a).

Grouping of Claims

For purposes of this appeal claims 32, 35-38, 91-93 and 95, directed to a diagnostic method, stand together.

For purposes of this appeal claims 46, 48-49, 52-59, and 94, directed to a diagnostic kit stand together.

Argument

1. Claims 32, 35-38, 46, 48, 52-59, 91-92 and 94 are directed to subject matter that is novel and not anticipated by Loizou et al .

The Examiner has stated that "the reference (Loizou et al) discloses an ELISA for measuring IgG and IgM anti-cardiolipin antibodies (ACA)". (Office Action dated 1/27/03, Paper No. 24 at page 2, No. 2.). The Examiner further stated that "this method of determining ACA levels is useful for clinical monitoring of patients with systemic lupus erythematosus (SLE) and associated immune disorders (abstract)". (*Id.*) Accordingly, the Examiner has asserted that "the method of the reference could be used with standard in stead of patient serum in order to create curves" and that "binding of the lipidic particles to the anti-lipidic particle [antibodies] occured in the presence of buffer." (*Id.*)

All the claims of the current application are directed to a kit or a method of assessing the amount of immune damage in a patient suffering from an autoimmune disease based on the presence of anti-lipidic particle antibodies and correlating such with an illness associated with antiphospholipid antibodies as in independent claims 32 and 46. The Loizou et al. reference does not anticipate the claims of the present application as the reference merely discloses an ELISA for measuring IgG and IgM anti-cardiolipin antibodies. The anti-cardiolipin antibodies disclosed

lipid particle

in the Loizou et al. reference are very different from the anti-lipidic particle antibodies specified in the present claims.

Anti-lipidic particle antibodies are not directed to an individual type of phospholipid but recognize a lipidic particle structure containing multiple different phospholipids. Phospholipids such as cardiolipin associated in lipidic particles in the lipid bilayers of liposomes or cells are the antigens used in this specification. In consequence, the monoclonal antibodies used in the present invention specifically react with phospholipids associated in lipidic particles in liposomes or cells, and they do not react with phospholipids associated in other molecular arrangements, such as liposomes or hexagonal II arrangements. In a similar way, the polyclonal antibodies detected in the present invention are antibodies against phospholipids associated in lipidic particles in liposomes or cells (Figure C).

The antigens used in the present invention are very different from the antigens as used in Loizou et al. In Loizou et al cardiolipin dissolved in ethanol is bound to ELISA polystyrene plates (Page 739, lines 46-48 of Loizou et al.) (Figure D). It is clear indeed that in such methodology there are no phospholipid bilayers nor any inductors for the formation of lipidic particles. In consequence, the antigens used by Loizou et al. are completely different from the antigens used in the present invention. It also is clear indeed that the antibodies that identify phospholipids bound to solid surfaces, as in Loizou et al, are completely different from the antibodies that identify phospholipids forming lipidic particles in liposomes or cells as set forth in the claims and described in the specification of the present invention. The anti-lipidic particle antibodies of the present invention are those antibodies that recognize the phospholipids in a particular structural environment different from the liposomes or hexagonal II arrangements. In

contrast, the antibodies according to Loizou et al are different because these recognize phospholipids such as cardiolipin in arrangements such as the liposome or hexagonal II arrangements. In particular, the present claims are directed to anti-lipidic particles antibodies, whereas Loizou et al. disclosed anti-cardiolipin antibodies.

Furthermore, Applicants submit that in Loizou et al. (1985) there is not any suggestion at all about phospholipids associated in lipidic particles, which are special molecular arrangements obtained only in lipid bilayers such as liposomes containing some phospholipids such as cardiolipin, the formation of which exclusively occurs in the presence of inductors. Thus, the antibodies in Loizou et al. are completely different from the anti-lipidic particle antibodies of the current claims. Therefore, determination of anti-cardiolipin by ELISA according to Loizou et al does not teach or suggest what is claimed in the present application.

Moreover, independent claims 32 and 46 of the present application make clear that the anti-lipidic particle antibodies are distinguished from anti-cardiolipin antibodies, as the method and kit allow for diagnosis of whether an individual is developing an illness associated with the presence of antiphospholipid antibodies, in circumstances where the individual does not present anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies. Therefore, the claims specifically distinguish the antibodies of the present invention and anti-cardiolipin antibodies of the Loizou et al reference.

In addition, the Examiner, in response to Applicants' previous arguments with respect to the reference, states that "the features upon which the applicant relies (i.e. one step method of carrying out the invention) are not recited in the rejected claim(s)." (Office Action dated 1/27/03, Paper No. 24 at page 5, No. 9.). However, the Examiner is mistaken because Applicants, in the

previous response, pointed out that the present claims require, as one step in carrying out the invention, the correlation of the presence of the amount of anti-lipidic particle antibodies. This limitation was included indeed as step (e) in claim 32, as amended. Applicants never made reference to a one step method.

Finally, the Examiner states that "the claims recite a correlation as one of the first events in illness. Therefore, read broadly, the claim merely requires that there be some correlation between the antibodies and illness at some point after illness onset, which the reference clearly discloses. The limitation of "one of the first events" does not substantially further limit the time period of illness correlation, as this terminology does not specify how early in the illness diagnosis must occur." (Office Action dated 1/27/03, Paper No. 24 at page 6, No. 10.)

In a first instance, it is respectfully pointed out that the reference neither teaches nor suggests a correlation between anti-lipidic particle antibodies and illness. As discussed, the reference discloses antibodies very different from the antibodies in the present invention. Furthermore, the reference contains no hint or even remote suggestion of a correlation with the first events of an illness in a human, which requires antibodies not even contemplated by this prior art. In addition, present claim 32 clearly limits the time period for which to correlate the presence of the antibodies of the present invention with illness. The early diagnosis may occur at any time between the detection of the anti-lipidic particle antibodies prior to the formation of anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies.

In addition to the above, the Loizou et al reference provides no suggestion whatsoever of the combination as claimed in the invention directed to a diagnostic kit. The kit comprises an antigen having lipidic particles, a buffer solution as medium to allow effective conditions of

binding the antigen to anti-lipidic particle antibodies in a sample, and a detectable-labeled reagent. The prior art only teaches methods employing anti-cardiolipin or antiphospholipid antibodies, and nothing in the prior art suggests a kit as in claims 46, 48, and 52-59.

Applicants thus respectfully submit that the claims 32, 35-38, 46, 48, 52-59, 91-92 and 94 of the present application contain subject matter that is novel and not anticipated by Loizou et al. Based on the foregoing, Applicants respectfully request withdrawal of this rejection.

2. Claims 32, 35-38, 46, 52-59, and 91-95 are directed to subject matter that is novel and not anticipated by Stewart et al .

The Examiner has stated that "the reference is disclosing a method for determining antiphospholipid antibodies in serum or plasma." (Office Action dated 1/27/03, Paper No. 24 at page 2, No. 3.). The Examiner further stated that "the antibodies have been implicated in various diseases including HIV". (Id. at page 3). According to the Examiner "in accordance with one aspect of the invention, polystyrene spheres are treated with a desired phospholipid to allow binding of the phospholipid thereto." (Id. at page 3) In addition, the Examiner asserts that "the reference also discloses the determination of antiphospholipid antibodies from the serum of healthy indiviuals." (Id. at page 3).

All the claims of the current application are directed to a kit or a method of assessing the amount of immune damage in a patient suffering from an autoimmune disease based on the presence of anti-lipidic particle antibodies, and correlating such with an illness associated with antiphospholipid antibodies, as set forth in independent claims 32 and 46. Stewart et al limits the measurement to individual arrangements of specific lipids, whereas the current claims require

that anti-lipidic particle antibodies interact with the lipidic particles that are being used for diagnosis, not with any phospholipid.

The Examiner states that Stewart et al discloses "in one embodiment, cardiolipin, which is a hexagon II lipid particle, is used as the phospholipid of the method (Col. 8, lines 4-11). The reference also discloses the determination of antiphospholipid antibodies from serum of healthy individuals (Col. 10, lines 49-54)." (Office Action dated 1/27/03, Paper No. 24 at page 3, No. 3.) In addition to the fact that cardiolipin may not be considered, in any way, as a hexagonal II lipid particle, applicants submit that in Stewart et al (1998) there is no mention of phospholipids associated in hexagonal II phase, which is a special tubular molecular arrangement of phospholipids obtained in an aqueous media only in the presence of divalent cations. Furthermore, in Stewart et al (1998) there is no suggestion at all of lipidic particles, which are special molecular arrangements obtained only in lipid bilayers such as liposomes containing some phospholipids (which may include cardiolipin), the formation of which occurs exclusively in the presence of inductors.

Anti-lipidic particle antibodies are <u>not</u> directed to an individual type of phospholipid, but recognize a lipidic particle structure containing multiple <u>different</u> phospholipids. Phospholipids such as cardiolipin associated in lipidic particles in the lipid bilayers of liposomes or cells, are the antigens used in this specification. In consequence, the monoclonal antibodies used in the present invention specifically react with phospholipids associated in lipidic particles in liposomes or cells, and they do not react with phospholipids associated in other molecular arrangements, such as liposomes or hexagonal II arrangements. In a similar way, the polyclonal antibodies

detected in the present invention are antibodies against phospholipids associated in lipidic particles in liposomes or cells (Figure C).

The antigens used in the present invention are very different from the antigens as used in Stewart et al. In Stewart et al polystyrene microspheres suspended in absolute ethanol (Col. 8, lines 24-25 and 50-54 of Stewart et al) are coated with cardiolipin dissolved in ethanol (Figure E). It is clear indeed that in such methodology there are no phospholipid bilayers nor any inductors for the formation of lipidic particles. In consequence, the antigens used by Stewart et al are completely different from the antigens used in the present invention. Furthermore, it is also clear indeed that the antibodies that identify phospholipids bound to solid surfaces (microspheres), as in Stewart et al, are completely different from the antibodies that identify phospholipids forming lipidic particles in liposomes or cells as required by the present claims and described in the specification of the present invention. Therefore, the anti-lipidic particle antibodies of the present claims are those antibodies that recognize the phospholipids in a particular structural environment different, from the liposomes or hexagonal II arrangements. In contrast, the antibodies according to Stewart et al are different because these recognize phospholipids such as cardiolipin in arrangements such as the liposome or hexagonal II arrangements. In particular, the present claims are directed to anti-lipidic particles antibodies, whereas Stewart et al discloses anti-cardiolipin and other anti-phospholipid antibodies. Therefore, determination of anti-phospholipid antibodies by using polystyrene microspheres treated with a particular phospholipid, according to Stewart et al dramatically differs from what is claimed in the present application.

In addition, the Examiner, in response to Applicants' previous arguments with respect to the reference, states that "the features upon which the applicant relies (i.e. one step method of carrying out the invention) are not recited in the rejected claim(s)." (Office Action dated 1/27/03, Paper No. 24 at page 5, No. 9.). However, the Examiner is mistaken because Applicants, in the previous response, pointed out that the present claims require, as one step in carrying out the invention, the correlation of the presence of the amount of anti-lipidic particle antibodies. This limitation was included indeed as step (e) in claim 32, as amended. Applicants never made reference to a one step method.

Further, the Examiner states that "the claims recite a correlation as one of the first events in illness. Therefore, read broadly, the claim merely requires that there be some correlation between the antibodies and illness at some point after illness onset, which the reference clearly discloses. The limitation of "one of the first events" does not substantially further limit the time period of illness correlation, as this terminology does not specify how early in the illness diagnosis must occur." (Office Action dated 1/27/03, Paper No. 24 at page 6, No. 10.)

Applicants respectfully point out that the reference neither teaches nor suggests a correlation between anti-lipidic particle antibodies and illness. As discussed, the reference discloses antibodies very different from the antibodies in the present invention. Furthermore, the reference contains no suggestion whatsoever of a correlation with the first events of an illness in a human, which requires antibodies in no way suggested by this prior art. In addition, present claim 32 clearly limits the time period for which to correlate the presence of the antibodies of the present invention with illness. The early diagnosis may occur at any time between the detection

of the anti-lipidic particle antibodies prior to the formation of anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies.

Also, the Examiner has stated that "the provision of lipids in their native states is advantageous, but has not stated in what manner." (Office Action dated 1/27/03, Paper No. 24 at page 6, No. 12.). In Applicant's previous response, Applicants referred to the fact that in Stewart et al, the phospholipids are bound directly to the solid phase of the microspheres, in a molecular arrangement substantially different from that found in cell membranes. According to the present claims, however, liposomes bearing lipidic particles are used to detect antibodies. Considering that liposomes are experimental models of cell membranes, they have the advantage of presenting lipids in a more native molecular arrangement (bilayer and lipidic particles), in contrast with phospholipid coated microspheres.

Finally, the Examiner stated that in response to Applicants' argument that "the antibodies in the present invention differ from Stewart is not convincing. It is ... noted that a reference is, in no way, limited only to its examples. Therefore, although the reference discloses specific particles, these are just preferred." According to the Examiner "although the antibodies between the present application and the reference may indeed be different, the claims require only anti-lipid particle antibodies, which the microspheres coated with antiphospholipid antibodies of Stewart is sufficient to meet." (Office Action dated 1/27/03, Paper No. 24 at page 6, No. 12.).

Applicants submit that in Figures C and E schematic representations of the antigens used in Stewart et al and the present invention are shown in comparison. In the present invention, lipids associated in lipidic particles in the bilayers of liposomes or cells are used as antigens (Figure C). In Stewart et al (1998), polystyrene beads coated with cardiolipin or other

phospholipids (Figure E) are used. In Figures C and E, it is shown that both antigens are clearly different from each other, in consequence antibodies against lipids associated in lipidic particles in liposomes or in cells are clearly different from antibodies against lipids bound to a solid polystyrene beads.

Applicants further submit the assertions by the Examiner are contrary to a careful reading of the prior art and the present application, because the claims of the present specification require anti-lipidic particle antibodies and lipidic particles in the bilayers of liposomes or cells as antigens. By no means are the microspheres coated with antiphospholipid antibodies, as suggested by the Examiner (but not by the prior art), sufficient to meet this requirement. In addition, the microspheres are coated with cardiolipin (Figure E) and these are used to identify anti-cardiolipin antibodies, instead of anti-lipidic particle antibodies as the present claims require.

In addition to the above, the Stewart et al reference does not teach or suggest the combination as claimed in the invention directed to a diagnostic kit. The kit comprises an antigen having lipidic particles, a buffer solution as medium to allow effective conditions of binding the antigen to anti-lipidic particle antibodies in a sample, and a detectable-labeled reagent. The prior art only teaches methods employing anti-cardiolipin or antiphospholipid antibodies, and nothing in the prior art suggests a kit as in claims 46, 52-59, and 94.

Applicants thus respectfully submit that the claims 32, 35-38, 46, 52-59, and 91-95 of the present application contain subject matter that is novel and not anticipated by Stewart et al. Based on the foregoing, Applicants respectfully request withdrawal of this rejection.

3. Claims 32, 35-38, 46, 48-49, 53-55, 59, and 91-95 are non-obvious over Ramirez et al (1994 and 1997) in view of Sugi et al .

The Examiner has stated that "Ramirez .. teach unilamellar liposomes formed from phosphatidylcholine:phosphatidate bearing lipidic particles induced by Mn ions, and polyclonal antibodies with reactivity toward these lipidic particles were produced, and monoclonal antibodies of the IgM isotype reacted with the lipidic particles." (Office Action dated 1/27/03, Paper No. 24 at page 4, No. 7.) According to the Examiner, it is disclosed that "the determination of antibodies to lipidic particles in the sera from patients of antiphospholipid syndrome and systemic lupus indicates that the lipidic particles induce production of antibodies." (Id. at page 5). According to the Examiner it also is taught that "the use of cardiolipin with their method, which is a hexagonal II lipidic particle." (Id. at page 5). In addition, the Examiner states that "the use of an ELISA procedure for the detection of antiphospholipid antibodies" is taught by Sugi et al. According to the Examiner it, would have been prima facie obvious ..... to use the microtiter plate taught by Sugi et al with the kit of Ramirez et al." (Id. at page 5).

In addition, the Examiner stated that "in response to applicant's argument that Ramirez et al. does not teach the diagnosis of an early phase of autoimmune disease, it is noted that this recitation leaves open at exactly what point in the disease state the correlation must be shown. The reference clearly teaches the determination of antibodies in patients with antiphospholipid syndrome and SLE with the lipid particles being present in the cell membranes of the patients." (Office Action dated 1/27/03, Paper No. 24 at page 6, No. 13.)

In contrast to the Examiner's assertions, a careful reading makes clear that the disclosure of Ramirez et al contains no hint or even remote suggestion that the antibodies obtained can be

used for the diagnosis of an early phase of an autoimmune disease. This prior art merely teaches the obtaining of the antibodies, and that the antibodies can be used to study the presence of lipidic particles in different cellular types. Sugi et al merely teaches the use of an ELISA procedure for the detection of antiphospholipid antibodies.

Furthermore, Ramirez et al do not provide any suggestion at all of a correlation between the detection of the antibodies and the first stages of an illness in a human. In the present claims, the presence of anti-lipidic particle antibodies in a sample from an individual is correlated with the first stages of primary antiphospholipid syndrome, or a disease associated with secondary antiphospholipid syndrome. Ramirez et al merely describe anti-lipidic particle antibodies. There is no hint therein, or even remote suggestion, of a correlation between the detection of the antibodies and the first stages of an illness in a human. A person having ordinary skill in the art at the time of the invention could not have predicted, based on the prior art, that detection of anti-lipidic particle antibodies could be correlated with the early stages of either primary antiphospholipid syndrome or a disease associated with secondary antiphospholipid syndrome.

In particular, the appearance of anti-lipidic particle antibodies, as well as anti-cardiolipin, anti-nuclear, anti-DNA and lupus anticoagulant antibodies, occur in a time period which is particular for each animal or human being, as this depends on the immune response of each individual. In consequence, it is difficult to limit the time period in which these antibodies appear. However, the formation of anti-lipidic particle antibodies always occurs before the formation of the anti-cardiolipin, anti-nuclear, anti-DNA and anti-coagulant antibodies, as shown in Figure F. This experimental information is not taught, mentioned nor suggested by Ramirez et

al. This prior art merely teaches the obtaining of the antibodies, and that the antibodies can be used to study the presence of lipidic particles in different cellular types. Therefore, Ramirez el al. in view of Sugi et al. does not disclose nor suggest the diagnostic method of the claimed invention, allowing early diagnosis of autoimmune diseases.

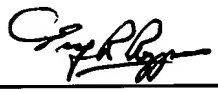
In addition to the above, the Ramirez et al (1994 and 1997) references in view of Sugi et al do not teach or suggest the combination as claimed in the invention directed to a diagnostic kit. The kit comprises an antigen having lipidic particles, a buffer solution as medium to allow effective conditions of binding the antigen to anti-lipidic particle antibodies in a sample, and a detectable-labeled reagent. Nothing in the prior art suggests a kit as in claims 46, 48-49, 53-55, 59, and 94.

Applicants thus respectfully submit that the claims 32, 35-38, 46, 48-49, 53-55, 59, and 91-95 of the present application are not obvious over Ramirez et al (1994 and 1997) in view of Sugi et al., Applicants respectfully request withdrawal of this rejection.

CONCLUSION

In view of the foregoing, Applicants respectfully submit that claims 32, 35-38, 46, 48, 52-59, 91-92 and 94 are novel and not anticipated under 35 U.S.C. § 102(b) by Loizou et al. In addition claims 32, 35-38, 46, 52-59, and 91-95 are novel and not anticipated under 35 U.S.C. §102(b) by Stewart et al. And finally, claims 32, 35-38, 46, 48, 52-59, 91-92 and 95 are non-obvious over Ramirez et al in view of Sugi et al.

All grounds for rejection of claims 32, 35-38, 46, 48-49, 52-59, and 91-95 are submitted to be unsupportable by the record, and thus improper. The Honorable Board is therefore respectfully requested to reverse the final rejection, and to direct the passage of this application to issue.

RESPECTFULLY SUBMITTED,					
NAME AND REG. NUMBER	George R. Repper, Registration No. 31,414				
SIGNATURE				DATE	7-28-03
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APPENDIX

32. A diagnostic method for indirectly determining the presence of lipidic particles in cell membranes from a sample suspected of having anti-lipidic particle antibodies from an individual suspected of suffering primary antiphospholipid syndrome or a disease associated with secondary antiphospholipid syndrome, wherein the presence of said lipidic particles in cell membranes allows diagnosis of whether said individual is developing an illness associated with the presence of antiphospholipid antibodies though said individual does not present anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies, comprising:

a) removing a sample suspected of having anti-lipidic particle antibodies from said individual, wherein said sample from said individual does not present anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies;

b) combining the removed sample with an antigen having said lipidic particles, said lipidic particles being immersed in a bilayer structure but not forming part of the bilayer structure, wherein said combining is under conditions effective to permit binding of anti-lipidic particle antibodies present in the sample to said antigen thereby forming a first mixture;

c) adding to the first mixture a detectable-labeled reagent useful for detecting binding of anti-lipidic particle antibodies to the antigen having lipidic particles thereby forming a second mixture;

d) detecting the presence of anti-lipidic particle antibodies in the sample bound to the antigen having lipidic particles in the second mixture, wherein said

detection of anti-lipidic particle antibodies bound to the antigens having lipidic particles is an indirect indication of the presence of lipidic particles in cell membranes of said individual; and

e) correlating the presence of anti-lipidic particle antibodies in the second mixture with immune damage in cell membranes having lipidic particles of said individual as one of the first events in illness associated with the presence of antiphospholipid antibodies.

35. The method of claim 32, wherein the detectable-labeled reagent comprises detectable-labeled polyvalent anti-human immunoglobulins second antibodies which bind to the anti-lipidic particle antibodies.

36. The method of claim 35, wherein the detectable-labeled anti-human immunoglobulin second antibodies comprises at least one anti-human immunoglobulin antibody directed against at least one human immunoglobulin class, and the presence of anti-lipidic particle antibodies is determined as one antibody selected from the group consisting of anti-lipidic particles IgG, IgM and IgA antibodies.

37. The method of claim 32, wherein the detectable-labeled reagent comprises one component selected from the group consisting of enzymes and fluorochromes, said component being attached to one element selected from the group consisting of polyvalent anti-immunoglobulins, anti-IgG, IgM and IgA immunoglobulin second antibodies.

38. The method of claim 32, wherein the detection of the presence of anti-lipidic particle antibodies in the sample from said individual is carried out using a protocol selected from the group consisting of ELISA, cytofluorometry and immunofluorescence.

46. A kit for use in an assay to indirectly determine the presence of lipidic particles in cell membranes from a sample suspected of having anti-lipidic particle antibodies from an individual suspected of suffering primary antiphospholipid syndrome or one disease associated with secondary antiphospholipid syndrome, wherein the presence of said lipidic particles in cell membranes allows diagnosis of whether said individual is developing an illness associated with the presence of antiphospholipid antibodies though said individual does not present anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies; comprising:

a) an indicator reagent comprising an antigen having lipidic particles to be contacted with the sample from said individual under conditions effective to permit binding of anti-lipidic particle antibodies present in the sample to the lipidic particles of the antigen, wherein said sample from said individual does not present anti-cardiolipin antibodies, lupus anti-coagulant, anti-DNA antibodies or anti-nuclear antibodies;

b) a buffer solution as a medium to allow effective conditions for the binding of the anti-lipidic particle antibodies present in the sample to the lipidic particles of the antigen; and

c) a detectable-labeled reagent useful for detecting the binding of anti-lipidic particle antibodies present in the sample to the lipidic particles of the antigen,

wherein the presence of the anti-lipidic particle antibodies in the sample can be correlated with immune damage in cell membranes having lipidic particles of said individual as one of the first events in illness associated with the presence of antiphospholipid antibodies.

48. The kit of claim 46, wherein said antigen comprises liposomes having lipidic particles induced with one agent selected from the group consisting of divalent cations and drugs producing lupus in humans, and wherein said liposomes are in one condition selected from the group consisting of liposomes bound to microtiter plates with a high lipidic binding property and liposomes suspended in an appropriate medium.

49. The kit of claim 46, wherein said antigen comprises neoplastic cells bound to one solid support selected from the group consisting of micro cover glasses and microtiter plates.

52. The kit of claim 46, wherein the buffer solution has a pH of 7.0 to 7.4.

53. The kit of claim 46, wherein the sample is selected from the group consisting of serum and plasma of said individual.

54. The kit of claim 46, wherein the detectable-labeled reagent comprises detectable-labeled polyvalent anti-human immunoglobulin second antibodies which bind to the anti-lipidic particle antibodies.

55. The kit of claim 54, wherein the detectable-labeled anti-human immunoglobulin second antibodies comprise at least one anti-human immunoglobulin antibody directed against at least one human immunoglobulin class and the presence of anti-lipidic particle antibodies is determined as an antibody selected from the group consisting of anti-lipidic particles IgG, IgM and IgA antibodies.

56. The kit of claim 46, wherein the detectable-labeled reagent comprises one component selected from the group consisting of enzymes and fluorochromes, said component being attached to one element selected from the group consisting of polyvalent anti-immunoglobulins, anti-IgG, IgM and IgA immunoglobulin second antibodies, and where the enzyme is selected from the group consisting of alkaline phosphatase and peroxidase, and the fluorochrome is selected from the group consisting of fluorescein isothiocyanate, phycoerythrin, Cy3 and Percp.

57. The kit of claim 46, further including a blocking solution to prevent false positive results from occurring when microtiter plates are used as a solid support, and at least a sample of a reference serum from a healthy individual as a negative control of the reaction with the antigen containing lipidic particles.

58. The kit of claim 46, further including at least an anti-lipidic particle polyclonal or monoclonal antibody to be reacted with the antigen having lipidic particles in order to confirm whether the anti-lipidic particle antibodies are present or not in said sample.

59. The kit of claim 46, wherein the detection of the presence of anti-lipidic particle antibodies in the sample from said individual is carried out using the protocol selected from the group consisting of liposomal-ELISA, cell-ELISA, immunofluorescence, liposomal-cytofluorometry and cell-cytofluorometry.

91. The method of claim 32, wherein removing a sample suspected of having anti-lipidic particle antibodies from said individual comprises removing an antibody porter.

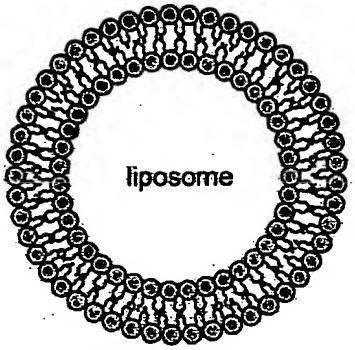
92. The method of claim 91, wherein the antibody porter comprises plasma or serum.

93. The method of claim 32 wherein said lipidic particles have a structural arrangement which is immersed in a bilayer structure of liposomes or cells without forming a part of said bilayer structure.

94. The kit of claim 47, wherein said antigen is selected from the group consisting of erythrocytes, leukocytes, and plaquettes, and said antigen is suspended in a medium consisting of a buffer solution that allows effective conditions for the binding of the anti-lipidic particle antibodies present in the sample to the lipidic particles of the antigen.

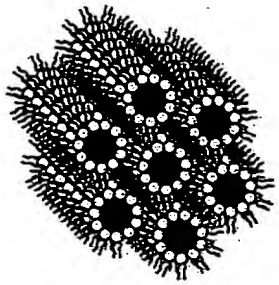
95. The method of claim 93 wherein the structural arrangement of said lipidic particles is selected from the group consisting of arrangements in hexagonal II and micellar phases.

Figure A



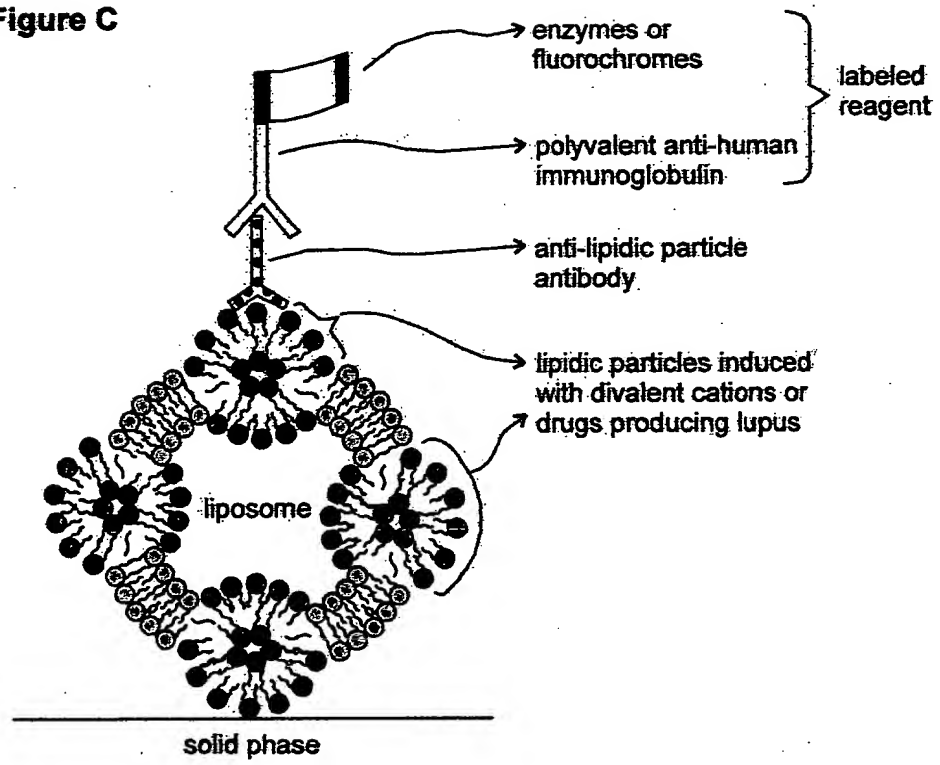
Cardiolipin in an aqueous media forming closed bilayer or liposomes.

Figure B



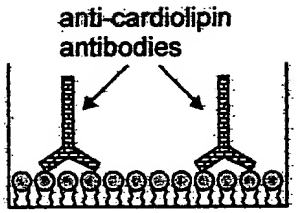
Cardiolipin in an aqueous media in the presence of the divalent cations Ca or Mn forming the hexagonal II phase.

Figure C



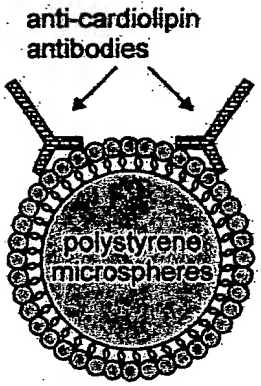
Cardiolipin forming liposomes with other phospholipids such as phosphatidylcholine in an aqueous media and in the presence of divalent cations or drugs producing lupus. The reaction of anti-lipidic particle antibody with the lipidic particle is ilustrated.

Figure D



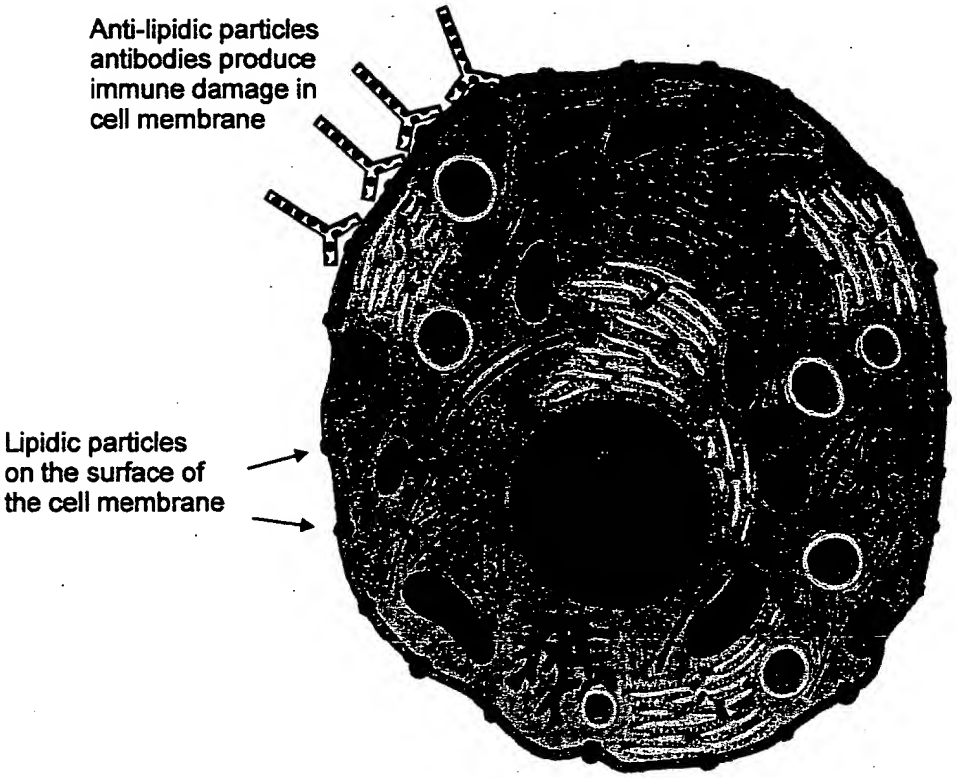
Probably molecular arrangement of cardiolipin dissolved in ethanol and bound directly on the solid polystyrene phase of the microtiter plate of ELISA, Loizou et al. (Clin. Exp. Immunol.1985). They do not use inductors of lipidic particles.

Figure E

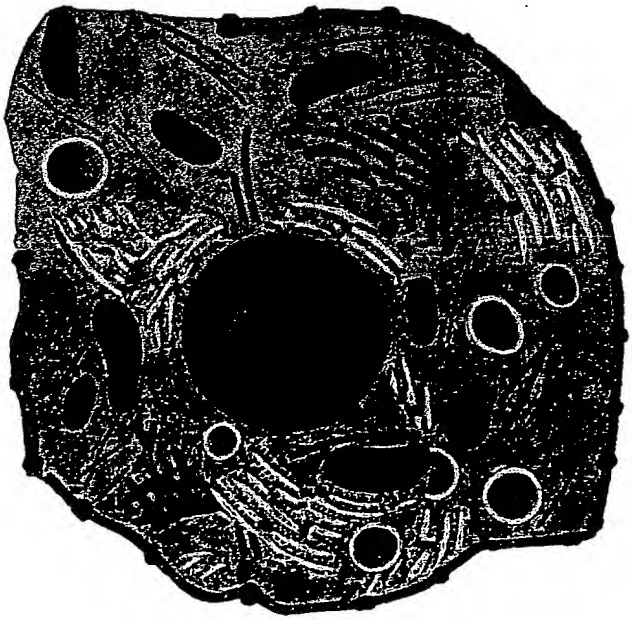


Probably molecular arrangement of cardiolipin dissolved in ethanol and bound to polystyrene microspheres from Stewart et al. (USA 5,840,587). They do not use inductors of lipidic particles.

Figure F



The binding of anti-lipidic particle antibodies to the lipidic particles on the cell membrane produces immune damage in the cell membrane that destroy the membrane exposing the intracellular components to the immunologic system and giving place later on to the formation of anti-cardiolipin, anti-nuclear, anti-DNA and anti-coagulant antibodies.



The cell is destroyed and the organelles are exposed to the immunologic system giving place to the formation of anti-cardiolipin antibodies, cardiolipin is a mitochondrial phospholipid, and anti-nuclear and anti-DNA antibodies.